

Evaluation of Some Cereals, Plants and Tubers Through Protein Composition

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Wild and cultivated maize, sorghum, rice, amaranth, soybean, and cassava were screened for variability in seed storage proteins. Total seed proteins, albumin (Alb-1 and Alb-2), globulin, alcohol-soluble (A1 and A2), and glutelin (G1 and G2) fractions were investigated by means of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The comparison was done by the obtained protein patterns and their relative amounts. Using quantitative analysis of the protein composition and the electrophoretic patterns, the relationships between total proteins and amount of individual proteins were determined. Electrophoretic patterns of extracted proteins from investigated samples showed that the main protein subunits were concentrated between 10 and 45 kDa. Variation was found in major fractions and minor bands. Electrophoretic patterns of the protein fractions are directly related to the genetic background of the protein and can be identified and used to certify the genetic makeup of wild, cultivated, or newly derived cereals and plants.

KEY WORDS: Plants; composition; proteins; electrophoresis.

1. INTRODUCTION

Certain of the cultivated and wild plants of Brazil, Colombia, and Mexico have unique or high protein composition. These plants show potential for beneficially altering the protein content and composition of animal feed, human food, and drinks upon selection following interbreeding and genetic manipulations (Paiva *et al.*,

1992; Vello *et al.*, 1988; Rodrigues, 1991). There is little quantitative information concerning their protein composition in terms of solubility classes of proteins and specific subunits.

Specific proteins observed during electrophoresis have been shown to be genetically coded on certain chromosomes. Since the genes responsible for storage protein synthesis are dominant and are all expressed in progeny, it is possible to follow the course of breeding experiments by electrophoretic analysis of protein extracts, which vary among species, and varieties of these cereals and plants (Rodrigues, 1991; Krishnan *et al.*, 1992; Wu, 1994; Higuchi and Fukazawa, 1987; Gorinstein, 1993; Gorinstein *et al.*, 1998). Proteins are an important group of biomacromolecules, which are involved in a variety of physiological functions, such as a dietary source of amino acids in foods (Gorinstein, 1993; Gorinstein *et al.*, 1998). Alcohol-soluble prolamins are the major storage proteins in such cereals as wheat, barley, rye, maize, and sorghum (Paiva *et al.*, 1992; Rodrigues, 1991; Gorinstein *et al.*, 1991a;

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El-Khalifa and El-Tinay, 1994). In some plants prolamins are not the storage proteins. Globulins are accumulated in oats, legumes, and tubers and glutelins in rice as their major storage proteins (Wu, 1994; Higuchi and Fukazawa, 1987; Wright, 1987; Gorinstein *et al.*, 1991b; Bressani and Garcia-Vela, 1990). It was shown that oat globulin and rice glutelin are highly homologous and are related to the legume 11S storage proteins (Okita *et al.*, 1988). Amaranth also has albumins and globulins as storage proteins (Gorinstein *et al.*, 1991b; Bressani and Garcia-Vela, 1990; Konishi *et al.*, 1991; Segura-Nieto *et al.*, 1994; Marccone *et al.*, 1994). Comparisons have been made among legumes, cereals, and tubers in relation to nutritional, structural, and functional properties (Peng *et al.*, 1981; Konishi and Yoshimoto, 1989; Kinsella and Phillips, 1989; Nagano *et al.*, 1994; Gorinstein *et al.*, 1996). To our knowledge there is no reference in the recent literature to the differences and identities of electrophoretic patterns in isolated seed protein fractions of amaranth, soybean, rice, sorghum, maize, and cassava.

This paper reports the distribution of proteins from different cereals and plants, as established by solvent extraction, and their identities and differences based on electrophoretic patterns of protein fractions.

2. MATERIALS AND METHODS

2.1. Sample Preparation

Whole mature seeds of the plants *Amaranthus* (*A.*) *caudatus* (yellow-brown) and *A. hypochondriacus* (yellow), two varieties of soybean with high and low oil contents (S-316 and S-804), cultivated and wild rice (*Oryza sativa* and *O. rufipogon*), roots of four types of wild cassava of different color (yellow, white, cream, and red; manioc, *Manihot esculenta*), maize (BR106-dent, CMS05-flint, and Pipoca-flint), and sorghum with high and low tannins (CMSXS114R with tannin about 1.21%, and CMSXS605R, with 0.54%) were investigated. All plants were grown in Brazil, except *A. caudatus* (Colombia) and *A. hypochondriacus* (Mexico).

Seeds of cereals and pseudocereals were ground on a mill through a 60-mesh screen. Roots of cassava were cut, lyophilized, and milled. The meal was defatted in a Soxhlet extractor with *n*-hexane for 10 hr and then was stored at 5°C after removal of hexane.

2.2. Protein Extraction

Total proteins were extracted directly from whole meal with 0.125 M Tris-HCl/5% SDS/2% 2-ME buffer,⁸ pH 6.8, with a solvent/sample ratio of (24/1 v/w). Samples were boiled for 5 min, cooled to room temperature, and centrifuged.

Proteins were also extracted stepwise according to the following methods (Paiva *et al.*, 1992; Gorinstein *et al.*, 1991a, b; Konishi *et al.*, 1991; Landry and Moreaux, 1970). The meal (1 g) was extracted with a solvent:sample ratio of 10:1 (v/w) and vigorously shaken. The extracts were separated by centrifuging at 10,000×g for 10 min. Each step was repeated twice. The sequence of the used solvents was the following: 0.5 M NaCl, water (albumin-1 and globulins); then water-albumin-2; 70% (v/v) isopropanol (IP)-prolamin A1; 70% (v/v) of IP, containing 4% (v/v) 2-mercaptoethanol (2-ME)-prolamin A2; 0.125 M sodium borate buffer, pH 10, containing 3% (v/v) 2-ME-glutelin G1 and then with the same solvent plus 0.5% (w/v) sodium dodecyl sulphate (SDS)-glutelin G2. Residual proteins were then extracted by 100 mM tris (hydroxymethyl) aminomethane (Tris)-glycine buffer, pH 7.5, containing 7.5% (v/v) 2-ME plus 5% (w/v) SDS. Extracts were combined, lyophilized, dissolved in sample buffer which contained 10% glycerol, 5% 2-ME, 2% SDS in 0.125 M (Tris-HCl), pH 6.8. Then the extracts were boiled for 5 min before being loaded. For all extracted samples proteins were then precipitated with acetone (1:2 vol) at -20°C overnight, and the precipitate was dissolved in the same sample buffer. The nitrogen content in each fraction was determined by the micro-Kjeldahl method combined with a colorimetric determination (Nkonge and Balance, 1982).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970), using both homogeneous 15% and 17% (w/v) gels. The gels were 1.5 mm thick and consisted of a 2-cm stacking gel and a 10-cm running gel. Fifty µg of protein was applied to sample slots.

Electrophoresis was carried out at 150 V for 3 hr. Gels were stained with 0.25% Coomassie brilliant blue R in methanol/water/acetic acid (5:5:1 v/v) and destained in the same solvent. Molecular weight standards were used to estimate protein subunit molecular

⁸ Abbreviations: BIS, N, N-methylene-bis-AA; kDa, kilodalton; 2-ME, 2-mercaptoethanol; MW, molecular weight; PAAG, polyacrylamide gel; IP, isopropanol; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TRIS, tris (hydroxymethyl)-aminomethane.

weights in kDa: hemoglobin, and cross-linked bovine (16, monomer; 32, dimer; 48, trimer; 64, tetramer).

3. RESULTS AND DISCUSSION

3.1. Protein Content

Total protein, albumin (Alb-1) and globulin (Glo), albumin-2 (Alb-2), alcohol-soluble (ASP) A1 and A2, glutelin (Glu) G1 and G2, and residual proteins from maize, high- and low-tannin sorghum, cultivated and wild rice, two varieties of amaranth, high- and low-oil soybean, and cassava roots were fractionated, as presented in Table I. The average proportions of Alb-1 and Glo, Alb-2, ASP A1, ASP A2, Glu G1, and Glu G2 in their nitrogen content in pseudocereals (amaranth and soybean) were 60.3:3.9:0.9:0.5: 5.0: 16.0, and in cereals (maize and sorghum) were 11:0.5:29:12:9:20. The obtained proportions for amaranth proteins in this study were similar to those for rice. The ratio of globulin to albumin in amaranth was about 2.1. Albumins, globulins, and glutelins in cereals represented about 84% of all nitrogen. Alcohol-soluble proteins (prolamin-like) A1 and A2 contained about 1.4% of the total nitrogen. This agrees with prolamin in amaranth not being a storage protein (Gorinstein *et al.*, 1991a,b; Bressani and Garcia-Vela, 1990; Konishi *et al.*, 1991; Marcone *et al.*, 1994). For maize, sorghum, and other cereals, however, prolamins contain about 42% of total nitrogen (Paiva *et al.*, 1992; Rodrigues, 1991; El-

Khalifa and El-Tinay, 1994). Some differences were also found (Table I) in the percentage distribution of protein fractions in sorghum with low and high tannin contents. The main storage protein was higher in low-tannin than in high-tannin sorghum, but the residual protein was higher in high-tannin than in low-tannin sorghum. The extraction of protein fractions from high-tannin sorghum was more complicated than from the low-tannin sorghum. This can be explained by the high amount of polyphenols, which formed a complex with the proteins. The protein content of Brazilian wild rice was higher than that of the cultivated rice. The distribution of the proteins in the wild rice differed in the alcohol-soluble fraction. The A2 and Glu G2 were higher for the wild sample than for the cultivated one. Probably wild rice has more S-S bridges than the cultivated rice, and therefore in fractions where 2-ME was used the amount of protein was higher. Extractability of alcohol-soluble proteins from seeds was studied at 20°C using IP mixtures with 2-ME varying from 0 to 5% with changes in extraction time, concentration of reducing agent, and proportion of solvent to solid (Gorinstein *et al.*, 1991a), mentioned in Section 2. After extraction of albumins and globulins the prolamins of all plants were extracted with a solvent (55% IP and 5% 2-ME) to sample ratio of 6:1 (optimum extraction conditions), as in previous studies (Gorinstein *et al.*, 1991a). The nitrogen content of alcohol-soluble proteins from cereals and other plants based on variable solvent systems used for extraction gave different results. The extraction depends on the milling of the

Table I. Nitrogen Content and Protein Distribution in Plants

| Plant ^a | Result for given protein fraction ^b | | | | | | | | |
|--------------------|--|------------|------------|------------|------------|-------------|------------|------------|---------|
| | Tot pro | Alb+Glo+P | Alb-2 | ASP A1 | ASP A2 | Glu G1 | Glu G2 | Residual | Rec (%) |
| Maize | 10.0 ± 0.8 | 9.1 ± 0.8 | 0.6 ± 0.05 | 32.4 ± 3.3 | 13.8 ± 0.9 | 11.2 ± 1.1 | 20.6 ± 3.1 | 13.1 ± 2.1 | 100.8 |
| Sorg. ht. | 9.7 ± 0.7 | 11.0 ± 0.9 | 0.2 ± 0.01 | 28.3 ± 3.2 | 12.2 ± 1.4 | 5.1 ± 0.3 | 20.1 ± 3.1 | 23.2 ± 2.8 | 100.1 |
| Sorg. lt. | 11.0 ± 0.9 | ±5.1 ± 1.2 | 0.9 ± 0.06 | 30.1 ± 3.3 | 13.4 ± 1.2 | 10.9 ± 0.8 | 19.4 ± 3.0 | 12.3 ± 1.1 | 100.1 |
| Rice os. | 8.0 ± 0.7 | 12.3 ± 1.1 | 1.2 ± 0.07 | 3.5 ± 0.2 | 1.7 ± 0.08 | 13.2 ± 1.2 | 55.3 ± 4.1 | 12.2 ± 1.1 | 99.4 |
| Rice or. | 13.0 ± 1.2 | 11.8 ± 1.1 | 0.9 ± 0.06 | 7.9 ± 0.7 | 6.5 ± 0.6 | 11.3 ± 1.1 | 50.8 ± 3.9 | 12.1 ± 1.1 | 101.3 |
| A. cau. | 16.6 ± 1.3 | 65.3 ± 4.4 | 3.7 ± 0.20 | 0.8 ± 0.1 | 0.4 ± 0.04 | 2.8 ± 0.1 | 13.2 ± 1.2 | 12.8 ± 1.2 | 99.1 |
| A. hyp. | 15.0 ± 1.2 | 57.3 ± 4.1 | 4.0 ± 0.30 | 0.9 ± 0.1 | 0.5 ± 0.04 | 7.1 ± 0.6 | 18.7 ± 1.3 | 12.1 ± 1.1 | 100.6 |
| Soya. ho. | 34.0 ± 3.3 | 56.3 ± 4.1 | 3.4 ± 0.20 | 7.2 ± 0.6 | 6.1 ± 0.4 | 4.5 ± 0.3 | 7.2 ± 0.6 | 16.3 ± 1.3 | 101.0 |
| Soya. lo. | 40.0 ± 3.5 | 70.0 ± 4.8 | 5.0 ± 0.40 | 2.4 ± 0.1 | 3.2 ± 0.2 | ± 3.5 ± 0.2 | 4.1 ± 0.3 | 13.2 ± 1.2 | 101.4 |
| Cassava, white | 3.0 ± 0.2 | 80.0 ± 5.1 | 1.8 ± 0.07 | 0.4 ± 0.03 | 0.7 ± 0.07 | 5.3 ± 0.4 | 3.3 ± 0.3 | 8.3 ± 0.7 | 99.8 |

^a Sorg. ht., sorghum, high tannin; Sorg. lt., sorghum, low tannin; Rice os, *Oryza sativa*; Rice or, *Oryza rufipogon*; A. cau, *Amaranthus caudatus*; A. hyp, *Amaranthus hypochondriacus*; Soya. ho, soybean, high oil; Soya. lo, soybean, low oil.

^b All results show mean values of triplicates ± standard deviation, expressed as percentage of nitrogen contained in the fraction to nitrogen contained in seeds. Tot pro, total proteins; Alb, albumins; Glo, globulins; P, nonprotein nitrogen; ASP, alcohol-soluble proteins; Glu, glutelins; Rec, recovery.

seed and sequence in which the solvents are used. Therefore only some data in Table I corresponded with the results of others (Paiva *et al.*, 1992; El-Khalifa and El-Tinay, 1994; Wright, 1987; Bressani and Garcia-Vela, 1990; Konishi *et al.*, 1991; Segura-Nieto *et al.*, 1994; Marcone *et al.*, 1994).

Figure 1A presents the main storage protein fractions for Alb, Glo, and Alb-2 for amaranth, soybean, and cassava, Glu G1 and Glu G2 for rice, and prolamins A1 and A2 for maize and sorghum.

3.2. Electrophoretic Separation

The separation of total proteins from cereals and plants is shown in Fig. 1B. Soybeans with high and low oil contents, S-316 and S-804 (Fig. 1B, lanes 2 and 3), were similar in electrophoretic profiles. Variety S-316 is a Brazilian grain-type soybean experimental line with a high oil content (27.6% against 21.0% in standard cultivars) with medium seed size (16 g/100 seeds). The cycle is semiearly, with 134 days from sowing to maturity. Toyohime (S-804) is an un-

adapted soybean genotype, a vegetable-type soybean with large seed size (40 g/100 seeds) introduced into Brazil from Japan, with chemical composition appropriate to making tofu with low oil content. The cycle is too early, with 100 days from sowing to maturity, like the check cultivar Williams. Two recurrent selection programs to enhance the genetic base (Vello *et al.*, 1988) of the soybean germplasm and to increase oil yield and improve seed character for human consumption (Vello *et al.*, 1988) were used for these samples. Two varieties of soybeans had shown the main subunits with the highest percentage of protein at 23 and 35 kDa. The subunits of high-tannin sorghum differed from low-tannin sorghum (Rodrigues, 1991; El-Khalifa and El-Tinay, 1994; Gorinstein *et al.*, 1996) in nearly all patterns. High-tannin patterns had less material than in low-tannin sorghum (Fig. 1B, lanes 4 and 5). The electrophoretic patterns of the storage proteins from the three varieties of maize were similar in their composition and relative amounts. Therefore only the results of Pipoca maize are shown in this study. Pipoca (Fig. 1B, lane 6) was rich in proteins in all main sub-

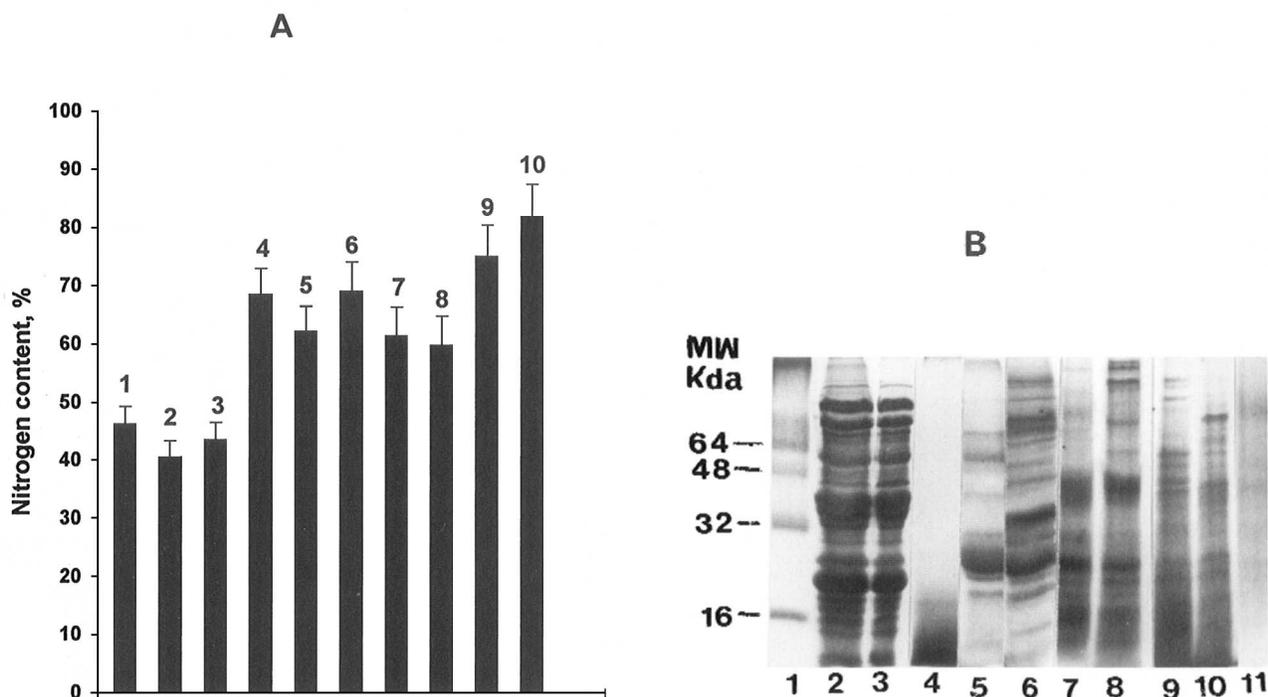


Fig. 1. (A) The main fraction of total seed proteins. (1) Maize Pipoca (maize-P.); (2) sorghum with high tannin content, (sorghum ht.); (3) sorghum with low tannin content, (sorghum lt.); (4) *Oryza sativa* (rice-os.); (5) *Oryza rufipogon* (rice or.); (6) *A. caudatus* (A. cau.); (7) *A. hypochondriacus* (A. hyp.); (8) soybean with high oil content (soya. ho.); (9) soybean with low oil content (soya. lo); (10) white cassava (cassava w). Mean \pm standard deviation (vertical lines). (B) SDS-PAGE of total seed proteins in 17% PAAG. (1) Molecular weight marker (kDa): hemoglobin, cross-linked bovine (16, monomer; 32, dimer; 48, trimer; 64, tetramer); (2) soya. ho; (3) soya. lo; (4) sorghum ht; (5) sorghum lt; (6) maize P; (7) rice os; (8) rice or; (9) A. cau; (10) A. hyp; (11) cassava w.

units and revealed similar patterns to low-tannin sorghum (Paiva *et al.*, 1992; Rodrigues, 1991). Two cultivars of rice (*Oryza sativa*) and (*Oryza rufipogon*) are shown in Fig. 1B, lanes 7 and 8, respectively. The main subunits in these two varieties were concentrated in the range between 10 and 45 kDa. Some slight differences were found in the minor fractions. *A. caudatus* and *A. hypochondriacus* (Fig. 1B, lanes 9 and 10) were similar in the main and minor subunits. *A. caudatus* differed from *A. hypochondriacus* mainly in the region 45–55 kDa, where especially *A. caudatus* showed some intense bands. Cassava (Fig. 1B, lane 11) showed very weak bands between 20 and 60 kDa, indicating a low amount of the protein and differences in comparison with other samples presented in Figs. 1A and 1B. Protein in cassava could be bound in inclusion bodies and thus be not easily extracted. Our results confirm that all proteinaceous material was extracted from particulate protein formations within these plant tissues.

As can be seen from presented electrophoretic patterns of different species and varieties of cereals and plants, protein subunits were concentrated between 10- and 50-kDa proteins. It is impossible to make a conclusion about the composition and quality of a specific variety only by electrophoretic patterns of total proteins. Therefore protein fractionation was used.

Albumins and globulins of soybean with low and high oil contents (Fig. 2A, lane 2) showed very similar patterns, with major two bands at 20 and 38 kDa and another two at 50 and 70 kDa with less material. Globulin fractions also were similar in their patterns and displayed major and average bands in the same range of molecular weight (Fig. 2A, lane 4). But the albumin-2 fraction differs from albumin-1 (Fig. 2A, lanes 3 and 5). Albumin-2, as mentioned in Section 2, was extracted with water after extraction of albumins and globulins (Konishi *et al.*, 1991). Alb-2 of low-oil soybean differs from Alb-1 and contained much more protein in the three subunits of 20, 38, and 50 kDa than Alb-1. Alb-2 of high-oil soybean differs from Alb-1, containing less protein material in the main subunits. The Alb-2 fraction extracted from amaranths was higher in comparison with that from maize and sorghum, but less than in the soybean fraction (Fig. 2A, lanes 6 and 7). Probably this fraction is associated with protein bodies because when the data of the present extraction were compared to the previous ones, only Glu G1 was reduced in amount. Alb-2 can be considered also as a part of the glutelin fraction.

Albumin and globulin fractions of two varieties of sorghum differed from the soybean fractions. In sorghum with low and high tannin content the amount

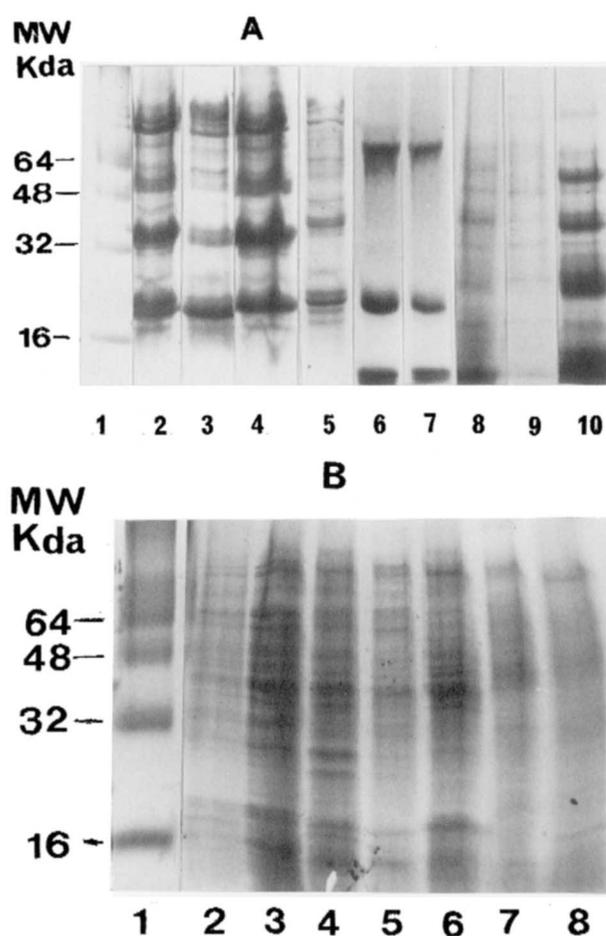


Fig. 2. (A) SDS-PAGE of water- and salt-soluble plant proteins in 15% PAAG. (1) Molecular weight marker; (2) (Alb-1 + Glo) from from soya. ho.; (3) Alb-1 from soya. ho.; (4) Glo from soya. ho.; (5) Alb-2 from soya. ho.; (6) (Alb-1 + Glo) from *A. hyp.*; (7) Alb-2 from *A. hyp.*; (8) Alb-1 from sorghum lt.; (9) Alb-2 from sorghum lt.; (10) Glo from sorghum lt. (B) SDS-PAGE of water- and salt-soluble plant proteins in 17% PAAG. (1) Molecular weight marker; (2) (Alb + Glo), cassava red; (3) Alb-1, cassava white; (4) Alb-1, cassava yellow; (5) Alb-1, cassava red; (6) Alb-1, cassava cream; (7) Glo, cassava white; (8) Glo, cassava yellow.

of Alb-1 was nearly the same. The amount of globulins was higher than the Alb-1 fraction and showed more intensive bands for low tannin (Fig. 2A, lane 10) than for high tannin content. The Alb-2 fraction in comparison with legumes was very low and the proteins were precipitated and concentrated to give some bands (Fig. 2A, lane 9). This means that the amount of Alb-2 fraction is higher in legumes and cereal-like plants (Fig. 2A, lanes 5 and 7) than in true cereals (Fig. 2A, lane 9). This correlates the Alb-2 fraction to plants in which albumins and globulins are the major storage proteins.

Four varieties of cassavas of different color (white, red, yellow, and cream) are shown on Fig. 2B, lanes 1–8. Albumins and globulins were are main protein fraction. The protein profiles are similar for all cassava varieties, showing many protein patterns concentrated between 16–20 and 30–80 kDa. The obtained profiles differ in the intensity of bands for different varieties (Fig. 2B, lanes 3 and 4, in comparison with lanes 5, 6).

Figure 3A shows the electrophoretic patterns of total alcohol-soluble proteins. Proteins from two varieties of soybeans with high and low oil contents, S-804 and S-316 (lanes 1 and 2), exhibit main electrophoretic bands at 16, 20, 32, 34, and 38 kDa. These two varieties were similar in profiles of their main bands. Total alcohol-soluble proteins from *A. caudatus* and *A. hypochon-*

driacus showed similar patterns at about 10–14 and 22 kDa (lanes 5 and 6). Sorghum CMSXS114R with low tannin content (lane 4) showed the main protein band at 23 kDa (Rodrigues, 1991). Cultivated and wild rice (lanes 8 and 9) showed a similarity in only one band of about 14 kDa. Sorghum with low tannin content, *A. caudatus*, and maize Pipoca had a similar band at 22 kDa (maize Pipoca was used as a standard for comparison having 19-, 22-, and 27-kDa bands). Patterns of alcohol-soluble protein (A1) from sorghum with low tannin showed three characteristic bands of 19 (very weak), 22, and 27 kDa. Sorghum CMSXS114R with high tannin was similar in alcohol-soluble protein A2 extracted with 70% IP and 4% 2-ME. But soybean alcohol-soluble fraction A2 revealed completely different bands, with major ones at 10, 29, 38, 42, and 48 kDa. Alcohol-soluble protein A1 (70% IP) from *A. caudatus* and *A. hypochondriacus* showed unseparated material of about 12 kDa and minor bands at 22 kDa and average ones at 48 kDa. ASP A2 from *A. caudatus* had one band at 18 kDa and another one at 44 kDa (data not shown).

Alcohol-soluble proteins of seeds extracted with 55% IP containing 5% 2-ME (6:1 v/w) from *A. caudatus* contain 80–85% of polypeptides of 10–14 kDa. The nonseparated proteins of subunits in the region of 8–14 kDa differ completely from those of other plants and cereals such as low- and high-tannin sorghum, rice, and maize (Gorinstein, 1993). The cereal prolamins did not show any electrophoretic relationship to amaranth or soybean alcohol-soluble fractions. The data proved that alcohol-soluble proteins (total, A1, and A2) could not represent the main protein fraction in cereal-like plants such as *A. caudatus*, *A. hypochondriacus*, soybean, and rice. The protein patterns of maize and sorghum (which were used only for comparison) showed that the alcohol-soluble proteins are the main storage proteins in these cereals.

Protein separation of glutelins G1 and G2 is shown in Fig. 3B. Subunits of 16, 30, and 50 kDa were in glutelins G1 (Fig. 3B, lane 1) and subunits of unseparated material at 10–14, 18, 22, and 28 kDa in glutelins G2 (Fig. 3B, lane 7) extracted from rice (*Oryza sativa*). Sorghum with low tannin (Fig. 3B, lane 2) showed 20, 33, and 49 kDa in their main subunits of glutelin G1. For sorghum with high tannin the amount of protein was very low. The detected minor bands appeared at 35 kDa (bands not shown). All protein subunits of glutelins G1 and G2, except only soybean with low oil content (Fig. 3B, lanes 3 and 9) and maize (Fig. 3B, lanes 6 and 12), had better separation when proteins were concentrated with acetone. Then the samples were applied to the gel. Such separation was better than the one which was di-

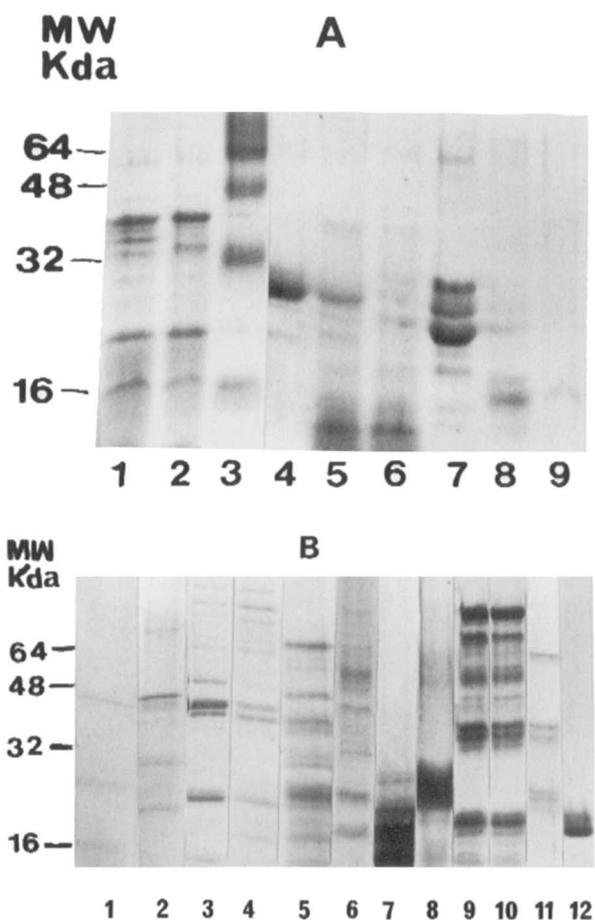


Fig. 3. (A) SDS-PAGE of total alcohol-soluble seed proteins in 17% PAAG. (1) soya. lo.; (2) soya. ho.; (3) standard; (4) sorghum lt.; (5) *A. cau.*; (6) *A. hyp.*; (7) maize P.; (8) rice os.; (9) rice or. (B) SDS-PAGE of glutelins from plant seeds in 15% PAAG. Glutelin G1 fraction: (1) rice os.; (2) sorghum lt.; (3) soya lo.; (4) soya ho.; (5) *A. cau.*; (6) maize P. Glutelin G2 fraction: (7) rice os.; (8) sorghum lt.; (9) soya lo.; (10) soya ho.; (11) *A. cau.*; (12) maize P.

rectly applied to the gel by mixing the protein extract with the sample buffer. Nonseparated material, which was mostly concentrated in the major fraction in the region between 20 and 28 kDa, was characteristic for glutelin G2 from sorghum with low tannin (Fig. 3B, lane 8). Glutelin G1 from soybean with low oil and from soybean with high oil content (Fig. 3B, lanes 3 and 4) showed three main subunits at 20, 35, and 50 kDa. Glutelin G2 from soybean with low oil content and soybean with high oil (Fig. 3B, lanes 9 and 10) had main subunits at 20, 50, and 75 kDa. Protein subunits of glutelins G1 and G2 for soybean with low oil (Fig. 3B, lanes 3 and 9) and high oil contents (Fig. 3B, lanes 4 and 10) display similar patterns, with major subunits at 20 and 40 kDa and average at 48, 64, and 85–100 kDa. The slight difference was only in minor bands; there were more for high oil than for low oil contents. The electrophoretic patterns of glutelin G1 from *A. caudatus* and *A. hypochondriacus* observed at 30, 45, 50, and 70 kDa were similar in protein content. Glutelin G2 of *A. caudatus* and *A. hypochondriacus* presented less protein material for *A. caudatus* in the region of 40 and 65 kDa than for *A. hypochondriacus* (Fig. 3B, lanes 5 and 11).

Electrophoretic patterns of residual proteins from three varieties of maize and two varieties of soybean showed that the extraction procedure employed in this study did not fractionate the proteins completely. The extract with 100 mM Tris, pH 7.5, 5% SDS, and 7.5% 2-ME completed extraction, but some repetition in the protein patterns could be seen.

This study has presented the results of extracting and characterizing the protein fractions from plant seeds. Some plants were evaluated for their main storage protein, in terms of both average proportions and relative amounts. Electrophoresis of the different protein classes in polyacrylamide gels on sodium dodecyl sulfate-containing media was used to separate and characterize individual proteins from different cereals and plants. It was shown that protein type can be established by extractions and electrophoretic analyses.

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